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APPLICATION NUMBER: *60/493,392*
FILING DATE: *August 07, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US03/34655*

By Authority of the
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PTO/SB/16 (10-01)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 302 339 378 US

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TITLE OF THE INVENTION (500 characters max)

USE OF AMINOCYCLOHEXYL ETHER COMPOUNDS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS (check all that apply)

[x] Specification Number of Pages	15	[ ] CD(s), Number	
[x] Drawing(s) Number of Sheets	6	[ ] Other:	
[x] Application Data Sheet. See 37 CFR 1.76 - 2 pgs			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

[ ] Applicant claims small entity status. See 37 CFR 1.27.

[ ] A check or money order is enclosed to cover the filing fees

[x] The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 03-1952

FILING FEE AMOUNT ($): $160.00

[ ] Payment by credit card. Form PTO-2038 is attached.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[x] No [ ] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EV 302 339 378 US in an envelope addressed to: Box Provisional Patent Application, Commissioner for Patents, Washington, DC 20231, on the date shown below.

Dated: August 7, 2003 Signature: _____ (LILIA OLSEN)

EV302339378US

16235 U.S. PTO 60/493392

Application Data Sheet - **ATTORNEY DOCKET: 554793000300**

Application Information

Application number::	Not Yet Assigned
Application Type::	Provisional
Subject Matter::	
Suggested Group Art Unit::	N/A
CD-ROM or CD-R?::	None
Sequence submission?::	None
Computer Readable Form (CRF)?::	No
Title::	
Attorney Docket Number::	554793000300
Request for Early Publication?::	No
Request for Non-Publication?::	No
Small Entity?::	No
Petition included?::	N/A
Secrecy Order in Parent Appl.?::	No

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Correspondence Customer Number::	20872

Representative Information

Representative Customer Number::	20872

Continuity Information NA

This application is a:
> Application One:
Filing Date:
which is a:

Prior Foreign Applications NA
Foreign Application One:
Filing Date:
Country:
Priority Claimed:

Use of Aminocyclohexyl Ether Compounds

Field of the Invention

This invention relates to the use of aminocyclohexyl ether compounds (e.g. as disclosed in PCT/CA99/00280) to block sodium current and therefore modulate class III-induced action potential prolongation and generation of triggered activity (EADs and TdP). Blockade of inward sodium currents by aminocyclohexyl ether compounds is thought to be the mechanism of action.

Background of the invention

Use of rabbit Purkinje fibers to screen for proarrhythmic activity:

Class III antiarrhythmics (I_{Kr} blockers) which have been shown to also be proarrhythmic cause greater lengthening in Purkinje fiber action potentials relative to those in ventricular muscle[1,2,3], presumably due to a greater contribution of I_{Kr} in repolarization of Purkinje fibers. In a cross species study, Lu et al.[4] found that dofetilide (10 nM) increased the APD_{90} of rabbit Purkinje fibers by 83%, versus 24% in the guinea pig, 65% in the dog, 18% in the pig, 61% in the goat and 30% in the sheep (BCL=1000 ms). Similarly, quinidine (10 μM) increased APD_{90} by 93% in the rabbit, versus 0% in the guinea pig, 16% in the dog, -3% in the pig, 0% in the goat and –24% in the sheep. In addition to drug induced dispersion of repolarization, drug induced EADs are thought to

[1] Antzelevitch C, Shimizu W, Yan GX, Sicouri S, Weissenburger J, Nesterenko VV, Burashnikov A, Di Diego J, Saffitz J, Thomas GP. The M cell: its contribution to the ECG and to normal and abnormal electrical function of the heart. J Cardiovasc Electrophysiol. 1999 Aug;10(8):1124-52.

[2] Abrahamsson C, Duker G, Lundberg C, Carlsson L. Electrophysiological and inotropic effects of H 234/09 (almokalant) in vitro: a comparison with two other novel IK blocking drugs, UK-68,798 (dofetilide) and E-4031. Cardiovasc Res. 1993 May;27(5):861-7.

[3] Abrahamsson C, Carlsson L, Duker G. Lidocaine and nisoldipine attenuate almokalant-induced dispersion of repolarization and early afterdepolarizations in vitro. J Cardiovasc Electrophysiol. 1996 Nov;7(11):1074-81.

[4] Lu HR, Marien R, Saels A, De Clerck F. Species plays an important role in drug-induced prolongation of action potential duration and early afterdepolarizations in isolated Purkinje fibers. J Cardiovasc Electrophysiol. 2001 Jan;12(1):93-102.

be an important cause of TdP both clinically[5] and in animal models[6]. Of the species tested, rabbit Purkinje fibers seem to be the most sensitive indicator of possible proarrhythmic activity in humans. Dispersion of repolarization and induction of EADs may be more prominent in rabbit Purkinje fibers relative to *in vivo* human Purkinje fibers, therefore any results should be interpreted with caution. Rather than as an appropriate model, rabbit Purkinje fibers should be considered a very sensitive test of I_{Kr} blockade, predicting possible proarrhythmic effects in a clinical setting.

Effects of Class I and class III agents in AF and EAD generation:

Class III agents have been shown to be proarrhythmic due to blockade of the hERG potassium channel (I_{Kr} current in human ventricle). It has been shown that combination therapy with quinidine (class III agent) and mexiletine (class I agent and sodium channel blocker) is more effective in the prevention of ventricular tachycardia (VT) and ventricular fibrillation (VF) in animal models[7,8] and in humans[9]. In isolated hearts, these effects have been shown to be due to sodium channel blockade[10]. EAD generation is thought to be a major cause of *torsades de pointes* (TdP) in humans. In addition, EADs have been shown to contribute to reinduction of atrial fibrillation (AF) following termination in isolated coronary-perfused canine right atria[11]. Sodium channel blockers have been shown to prevent isoproterenol-induced TdP in a canine model and also

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[5] Carlsson L, Amos GJ, Andersson B, Drews L, Duker G, Wadstedt G. Electrophysiological characterization of the prokinetic agents cisapride and mosapride in vivo and in vitro: implications for proarrhythmic potential? J Pharmacol Exp Ther. 1997 Jul;282(1):220-7.

[6] Vos MA, Verduyn SC, Gorgels AP, Lipcsei GC, Wellens HJ. Reproducible induction of early afterdepolarizations and torsade de pointes arrhythmias by d-sotalol and pacing in dogs with chronic atrioventricular block. Circulation. 1995 Feb 1;91(3):864-72.

[7] Duff HJ, Gault NJ. Mexiletine and quinidine in combination in an ischemic model: supra-additive antiarrhythmic and electrophysiologic actions. J Cardiovasc Pharmacol. 1986 Jul-Aug;8(4):847-57.

[8] Duff HJ. Mexiletine-quinidine combination: enhanced antiarrhythmic and electrophysiologic activity in the dog. J Pharmacol Exp Ther. 1989 May;249(2):617-22.

[9] Duff HJ, Mitchell LB, Manyari D, Wyse DG. Mexiletine-quinidine combination: electrophysiologic correlates of a favorable antiarrhythmic interaction in humans. J Am Coll Cardiol. 1987 Nov;10(5):1149-56.

[10] Duff HJ, Cannon NJ, Sheldon RS. Mexiletine-quinidine in isolated hearts: an interaction involving the sodium channel. Cardiovasc Res. 1989 Jul;23(7):584-92.

[11] Burashnikov A, Antzelevitch C. Reinduction of atrial fibrillation immediately after termination of the arrhythmia is mediated by late phase 3 early afterdepolarization-induced triggered activity. Circulation. 2003 May 13;107(18):2355-60.

abbreviate action potential duration in M-cells of the ventricular myocardium[12]. Another study has shown that mexiletine in the canine arterially perfused left ventricular wedge preparation reduced transmural dispersion of repolarization and suppressed EADs induced by ATX-II and d-sotalol[13]. This action of mexiletine is thought to be due to the abbreviation of M-cell action potential duration.

Role of the sodium channel in action potential:

High densities of voltage-gated sodium channels in excitable tissues lead to a rapid membrane depolarization when excitable cells reach the threshold for sodium channel activation. The role of sodium channels in the action potential upstroke (Phase 0) has been well-characterized and block of sodium channels can affect cellular refractoriness and regulate heart rhythms. Sodium channels rapidly inactivate following initial opening during phase 0 and during repolarization recovery of these inactivated channels is critical in determining the ability of a cell to generate another action potential. The period during which the cell cannot generate another action potential is known as the effective refractory period (ERP). Blockade of sodium channels can lengthen the refractory period of the cell and this activity is known to have antiarrhythmic consequences due to prolongation of the effective wavelength of the tissue, reducing the size of reentrant wavelets which the tissue can support[14]. Blockade of sodium channel can also suppress ectopic beats which may also play a role in the genesis of fibrillatory activity in the heart. Indeed, the selective sodium channel blocker tetrodotoxin (TTX) has been shown to prevent VF in isolated rabbit hearts[15]. Recent evidence has shown that sodium channel activity contributes not only to the action potential upstroke, but also can affect the action potential plateau (Phase 2) and repolarization (Phase 3). This sustained activity is

[12] Shimizu W, Antzelevitch C. Cellular basis for the ECG features of the LQT1 form of the long-QT syndrome: effects of beta-adrenergic agonists and antagonists and sodium channel blockers on transmural dispersion of repolarization and torsade de pointes. Circulation. 1998 Nov 24;98(21):2314-22.

[13] Sicouri S, Antzelevitch D, Heilmann C, Antzelevitch C. Effects of sodium channel block with mexiletine to reverse action potential prolongation in in vitro models of the long term QT syndrome. J Cardiovasc Electrophysiol. 1997 Nov;8(11):1280-90.

[14] Nattel S. New ideas about atrial fibrillation 50 years on. Nature. 2002 Jan 10;415(6868):219-26.

[15] Duff HJ, Sheldon RS, Cannon NJ. Tetrodotoxin: sodium channel specific anti-arrhythmic activity. Cardiovasc Res. 1988 Nov;22(11):800-7.

thought to be a result of 3 separate mechanisms. The first of such mechanisms has been described as channel bursting in which the channel fails to inactivate[16]. A second component is known as window current and occurs at potentials at which the steady-state activation and inactivation curves overlap[17]. The third mechanism is a non-equilibrium phenomenon in which the sodium channels recover from inactivation during the repolarization phase[18]. The sustained inward sodium current contributed by these three mechanisms can modulate repolarization during phase 2 and phase 3 of the action potential when the membrane potential is regulated by small amounts of both inward and outward current. Modulation of currents contributing to phase 0, 2 and 3 of the action potential can have important roles in regulating refractoriness, action potential duration and EAD generation.

Brief description of the invention:

The present invention concerns the perfusion of aminocyclohexyl ether compounds (e.g. RSD1235) at concentrations sufficient to block sodium current either before or during perfusion with proarrhythmic agents (e.g. dofetilide or other class III agents) in order to attenuate action potential prolongation and/or EAD generation which are known to have proarrhythmic consequences. Sodium channel blockade by aminocyclohexyl ether compounds (e.g. RSD1235) can prevent induction of AF or VF as well as terminate triggered activity which is thought to lead to fatal VF. The invention relates to the effects of aminocyclohexyl ether compounds (e.g. RSD1235) in rabbit Purkinje fibers, but the invention can likely be extended to treatment of acquired long-QT syndrome, muti-focal ventricular arrhythmias (TdP) or prevention of AF induction in humans.

[16] Bennett PB, Yazawa K, Makita N, George AL Jr. Molecular mechanism for an inherited cardiac arrhythmia. Nature. 1995 Aug 24;376(6542):683-5.
[17] Wang DW, Yazawa K, George AL Jr, Bennett PB. Characterization of human cardiac Na+ channel mutations in the congenital long QT syndrome. Proc Natl Acad Sci U S A. 1996 Nov 12;93(23):13200-5.
[18] Clancy CE, Tateyama M, Liu H, Wehrens XH, Kass RS. Non-equilibrium gating in cardiac Na+ channels: an original mechanism of arrhythmia. Circulation. 2003 May 6;107(17):2233-7.

Detailed description of methods:

Action Potential duration measurements:

Female, white New Zealand rabbits weighing between 2.5 and 3.5 kilograms are anaesthetized with a sufficient dose of pentobarbital to create a stuporous state and the animals are sacrificed with a blow to the head. A midline thoracotomy is performed and the heart is excised as is practised by those skilled in the art. The right and left atrium and removed and the heart is opened through an incision along the left side of the septum in order to expose the endocardial surface of the left ventricle. The heart is transferred to a 10 mL tissue bath and Purkinje fibers are exposed for microelectrode recording. The heart is perfused with standard bicarbonate buffered Krebs' solution known to those skilled in the art. An electrode is pulled from thin-walled filamented borosilicate glass capillary tubes having a resistance of 10 to 30 megaohms when filled with 3 M KCl. The electrode is attached to headstage mounted on an Axoclamp 2A amplifier or a similar amplifier known to those skilled in the art. The microelectrode is brought down upon an exposed Purkinje fiber using a micromanipulator and the position is adjusted until the electrode penetrates a single Purkinje cell. The Purkinje fiber network is stimulated using a biphasic stimulation pulse and subsequent action potentials are recorded for analysis. Extracellular solutions containing aminocyclohexyl ether compounds (e.g. RSD1235) and/or proarrhythmic agents (e.g. dofetilide or other class III agents) and then perfused in order to discern changes in action potential duration. A dose response relationship is obtained using ascending concentrations of aminocyclohexyl ether compounds (e.g. RSD1235) (0.3 to 30 μM) and this treatment is then followed by concomitant perfusion with 300 nM proarrhythmic agents (e.g. dofetilide or other class III agents) and 30 μM aminocyclohexyl ether compounds (e.g. RSD1235). In a separate preparation, a dose-response relationship is obtained using ascending concentrations of proarrhythmic agents (e.g. dofetilide or other class III agents) (10 nM to 300 nM) and this treatment is followed by concomitant perfusion with 300 nM proarrhythmic agents (e.g. dofetilide or other class III agents) and 30 μM aminocyclohexyl ether compounds (e.g. RSD1235). A final study is undertaken in which ascending concentrations of proarrhythmic agents (e.g.

dofetilide or other class III agents) is paired with DMSO vehicle control, 30 μM aminocyclohexyl ether compounds (e.g. RSD1235) or 100 μM lidocaine, the identity of which is blinded to the experimenter.

Effective Refractory Period (ERP) measurements:

In the same preparations as described above, ERP is determined following each treatment arm. An S1-S2 protocol is used as known by those skilled in the art. Briefly, 15 S1 pulses are delivered at a frequency of 1 Hz and this train is followed by an S2 pulse following a variable interval. The interval is set to be greater than the refractory period and it is reduced in 10 ms increments until an S2 response can no longer by elicited. The shortest duration which can generate an S2 response is termed the ERP.

Early-after-depolarization (EAD) measurements:

The left ventricle of a rabbit heart is exposed as described previously. A Purkinje fiber is located within the Purkinje fiber network having dimensions of approximately 2 mm length and 0.5 mm width. The fiber is excised from the heart using fine cutting tools along with a small amount of ventricular tissue attached at either end of the fiber. The fiber is transferred to a 5 mL tissue bath and perfused and penetrated as described above. Stable action potentials are obtained for a period not less than 30 minutes and then 300 nM proarrhythmic agent (e.g. dofetilide or other class III agents) is perfused in order to generate EADs. EADs are characterized as depolarizations which disrupt the normal course of Purkinje fiber repolarization. Stable EADs are obtained for a period of not less than 30 minutes and then 30 μM aminocyclohexyl ether compounds (e.g. RSD1235) is perfused concomitantly with 300 nM proarrhythmic agent (e.g. dofetilide or other class III agents). EADs are monitored for termination over a period not exceeding 60 minutes.

Detailed description of results:

The initial experiments explored the effect of a single concentration of RSD1235 (30 μM) following ascending concentrations of dofetilide and vice versa (single concentration of dofetilide was 300 nM). Figure 1 illustrates the changes in action potential duration following these various treatments. The change in APD_{50} during RSD1235 treatment was not significant ($p > 0.05$), however immediately following perfusion with 30 μM RSD1235, concomitant perfusion of 30 μM RSD1235 and 300 nM dofetilide induced a 20% increase in APD_{50} ($p < 0.01$) (Fig. 1A). This effect of the combination of RSD1235 and dofetilide was much less than the increase observed with 300 nM dofetilide alone which produced approximately a 100% increase in APD_{50} ($p < 0.01$) (Fig. 1B). Subsequent treatment with 300 nM dofetilide and 30 μM RSD1235 produced a reduction in APD_{50} to 70% of the APD_{50} for dofetilide alone (Fig. 1B). It appears that pretreatment with ascending concentrations of RSD1235 (Fig. 1A) reduced the effect of dofetilide more than acute treatment (Fig. 1B). Similar, but less pronounced effects upon APD_{90} were observed (Figs. 1C & D). As reported earlier (report EP020117-01), RSD1235 alone produced only mild increases in the PF APD_{90} at concentrations up to 30 μM (Fig 1C). Concomitant treatment with 300 nM dofetilide and 30 μM RSD1235 induced a 60% increase in APD_{90} (Fig. 1C) which was less than the 105% increase in APD_{90} caused by 300 nM dofetilide alone (Fig. 1D). Similar to the effects observed on APD_{50}, RSD1235 treatment following dofetilide pretreatment did not reduce the effects of dofetilide upon APD_{90} (105% increase) as much as dofetilide treatment following RSD1235 pretreatment (60% increase) (Fig. 1D).

Whereas RSD1235 tended to mildly attenuate the prolongation of APD_{50} and APD_{90} induced by dofetilide (Fig. 1B & D), RSD1235 tended to prolong the PF ERP produced by dofetilide (Fig. 2). In addition, whereas pretreatment with RSD1235 significantly reduced the subsequent effect of dofetilide upon APD_{90} and APD_{50}, RSD1235 pretreatment did not attenuate ERP prolongation induced by dofetilide (Fig. 2 and 3).

The second set of experiments illustrated in figures 4 & 5 explored the effects of concomitant RSD1235/dofetilide treatment over the entire dose range of dofetilide (10 to 300 nM). Blinded to the experimenter, either 30 μM RSD1235 or DMSO was perfused with dofetilide. Concomitant treatment with 30 μM RSD1235 significantly blunted the effects of dofetilide upon APD_{50} (Fig. 4) and APD_{90} (Fig. 5) when compared to the vehicle control (DMSO). The percentage increase in APD_{50} induced by 300 nM dofetilide in the presence of DMSO or 30 μM RSD1235 was 86 and 37% respectively while the increase in APD_{90} was 92% and 61% respectively. There was no significant depression of V_{max} in the presence of dofetilide and RSD1235 relative to dofetilide and DMSO (205 ±37 V/s and 253 ±48 V/s respectively, n=10, p=0.44).

The reduction in dofetilide-induced APD_{50} prolongation in the presence of 30 μM RSD1235 suggests that 30 μM RSD1235 may be effective in the termination of dofetilide-induced early-after-depolarizations (EADs) in isolated rabbit Purkinje fibers. Preliminary data (Fig. 6) shows dofetilide induced EADs (B & C) in an isolated Purkinje fiber preparation. Stable EADs are obtained after approximately 30 minutes of perfusion with 300 nM dofetilide. Subsequent perfusion with 30 μM RSD1235 and 300 nM dofetilide produces a time-dependent reduction in the number of early-depolarizations (D & E) and after approximately 10 minutes of RSD1235 perfusion, complete abolishment of any EAD activity (F). The termination of EADs by 30 μM RSD1235 was seen in 4 of 4 preparations studied.

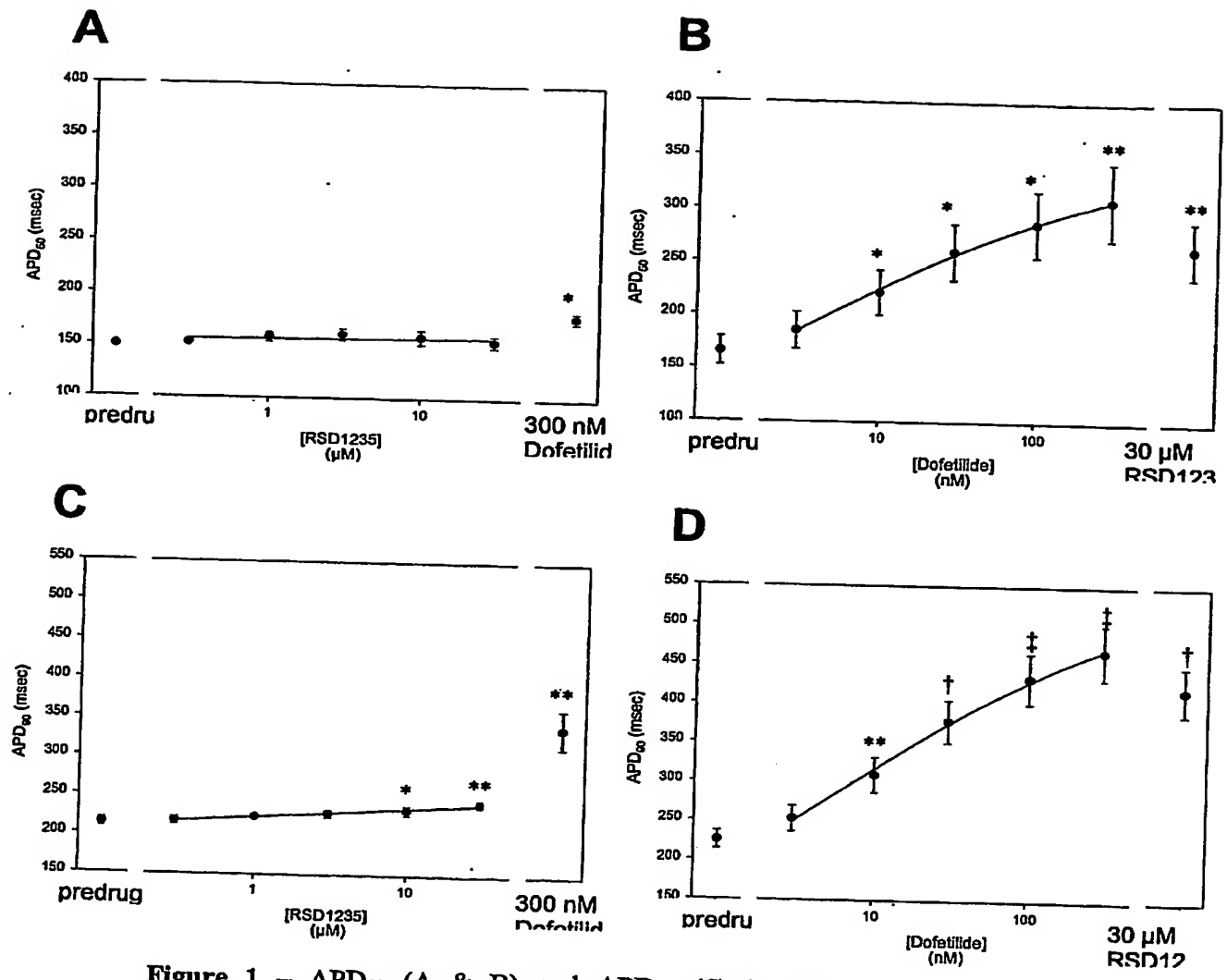


Figure 1 – APD_{50} (A & B) and APD_{90} (C & D) in the presence of escalating concentrations of RSD1235 (A & C) or dofetilide (B & D) followed by perfusion with RSD1235 (30 μM) and dofetilide (300 nM). The stimulation frequency was 1 Hz and results are expressed as the mean ±S.E.M., (n=4). * $p < 0.05$, ** $p < 0.01$, † $p < 0.001$, ‡ $p < 0.0001$ relative to predrug values.

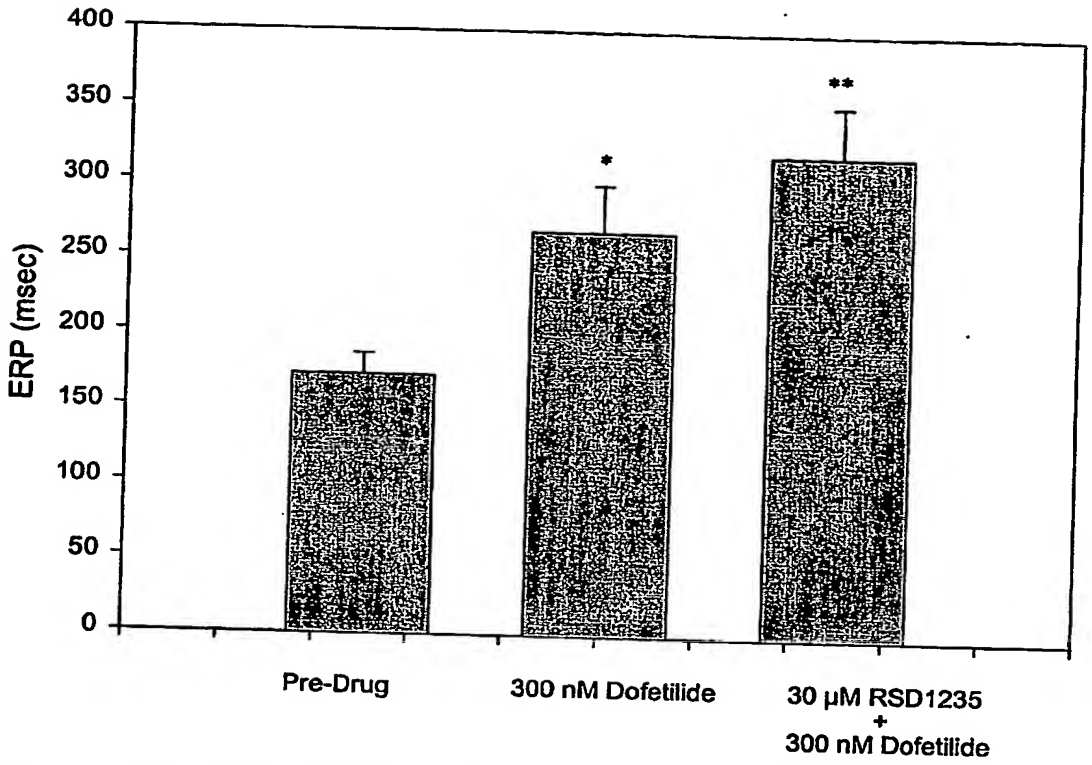


Figure 2 – ERP values obtained in the presence of dofetilide or a combination of dofetilide and RSD1235. The S1-S1 interval was 1 second and 8 S1 pulses preceded the S2 pulse. Data are expressed as mean values ±S.E.M., n=4. * $p < 0.05$ and ** $p < 0.01$ relative to predrug.

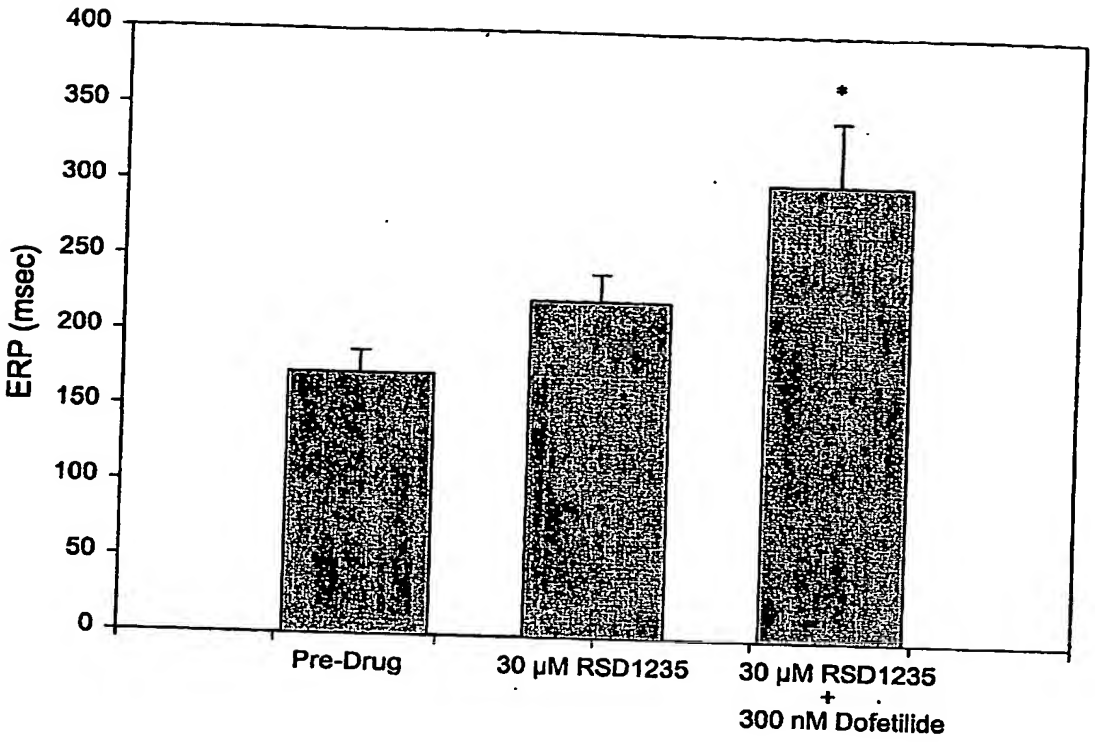
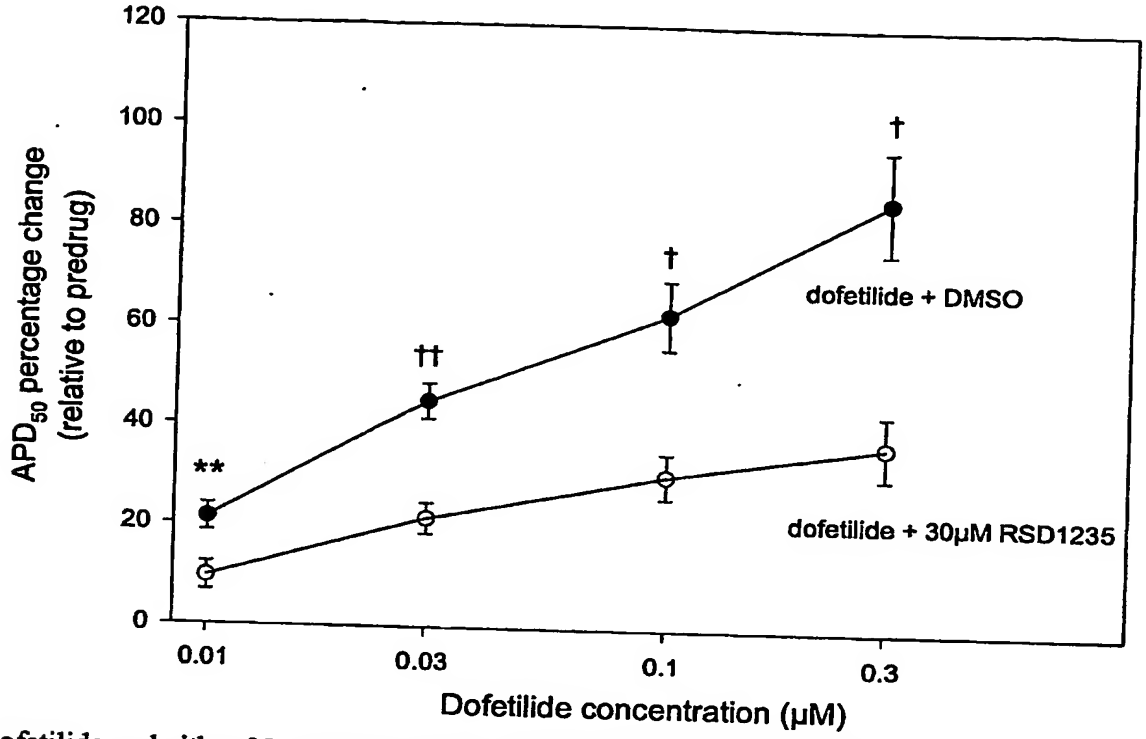


Figure 3 – ERP values obtained in the presence of RSD1235 or a combination of RSD1235 and dofetilide. The S1-S1 interval was 1 second and 8 S1 pulses preceded the S2 pulse. Data are expressed as mean values ±S.E.M., n=4. * $p < 0.05$ relative to predrug.

Figure 4 – Percent increases in APD_{50} when Purkinje fibers were co-treated with



dofetilide and either 30 μM RSD1235 or DMSO control. The stimulation frequency was 1 Hz. Results are expressed as the mean ±S.E.M. (n=10). ** $p < 0.01$, † $p < 0.001$ and †† $p < 0.0001$ for RSD1235 cotreatment relative to vehicle control.

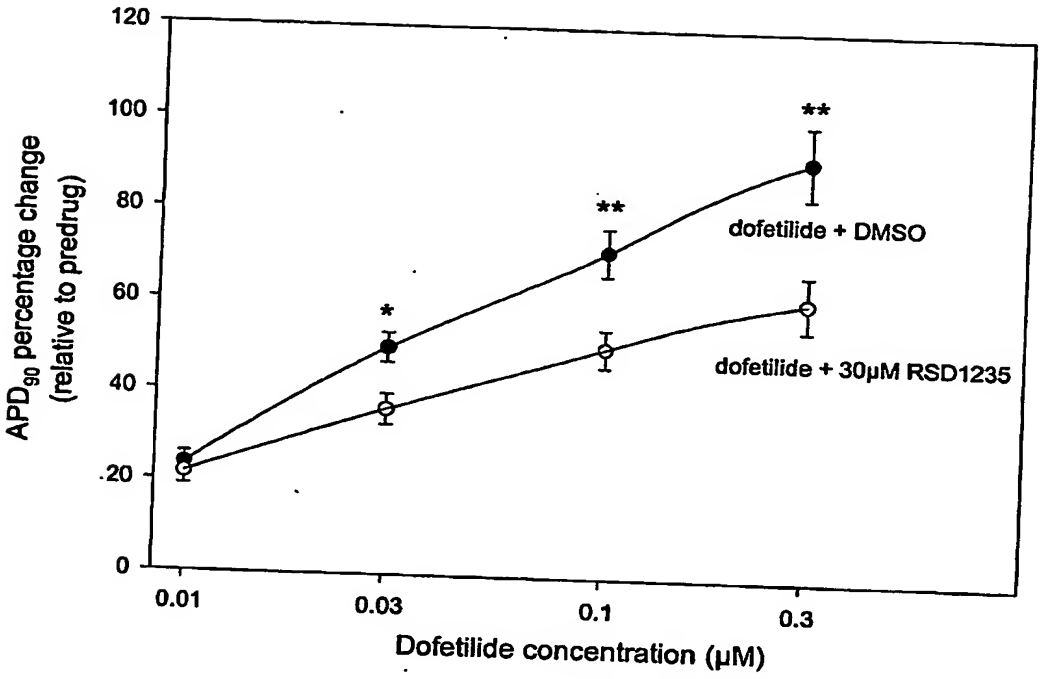


Figure 5 – Percent increases in APD_{90} when Purkinje fibers were co-treated with dofetilide and either 30 μM RSD1235 or DMSO control. The stimulation frequency was 1 Hz. Results are expressed as the mean ±S.E.M. (n=10). * $p < 0.05$, ** $p < 0.01$ for RSD1235 cotreatment relative to vehicle control.

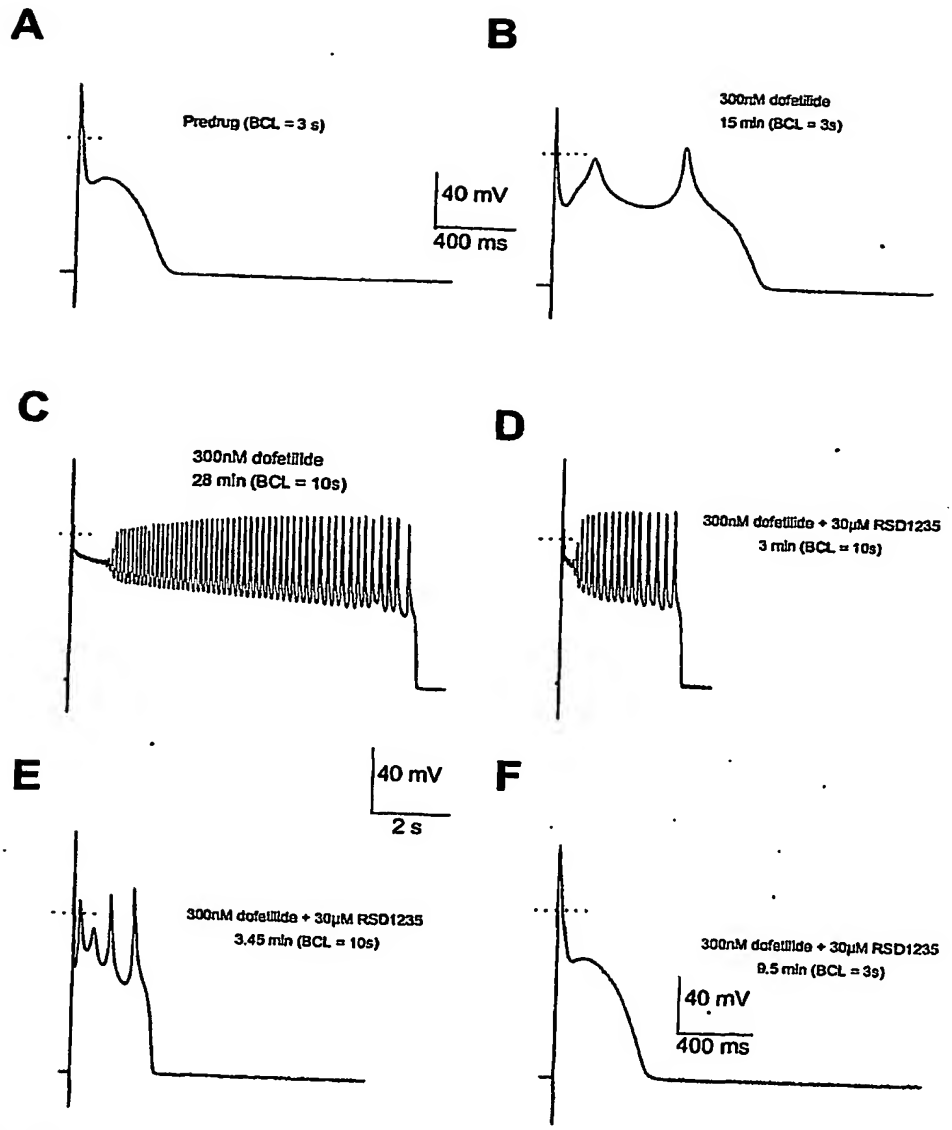


Figure 6 - Termination of dofetilide-induced EADs by RSD1235 in isolated Purkinje fibers. Cycle length and treatment conditions are indicated in the text of each panel. Panels A, B and F are on a more expanded time base than panels C, D and E (see scale bars). Zero millivolts is indicated by the dotted line in each panel.

All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, or patent application were specifically and individually indicated to be so incorporated by reference.

sf-1547677